

# An Automated High-Throughput Extraction and Real-Time PCR Solution for Rapid Mpox Detection

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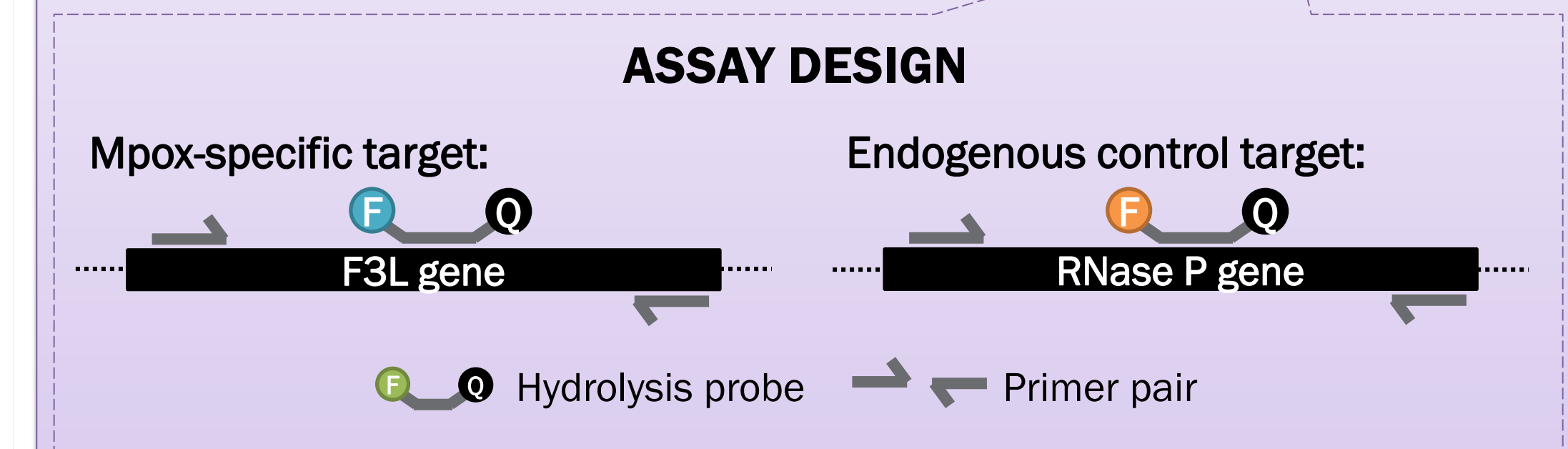
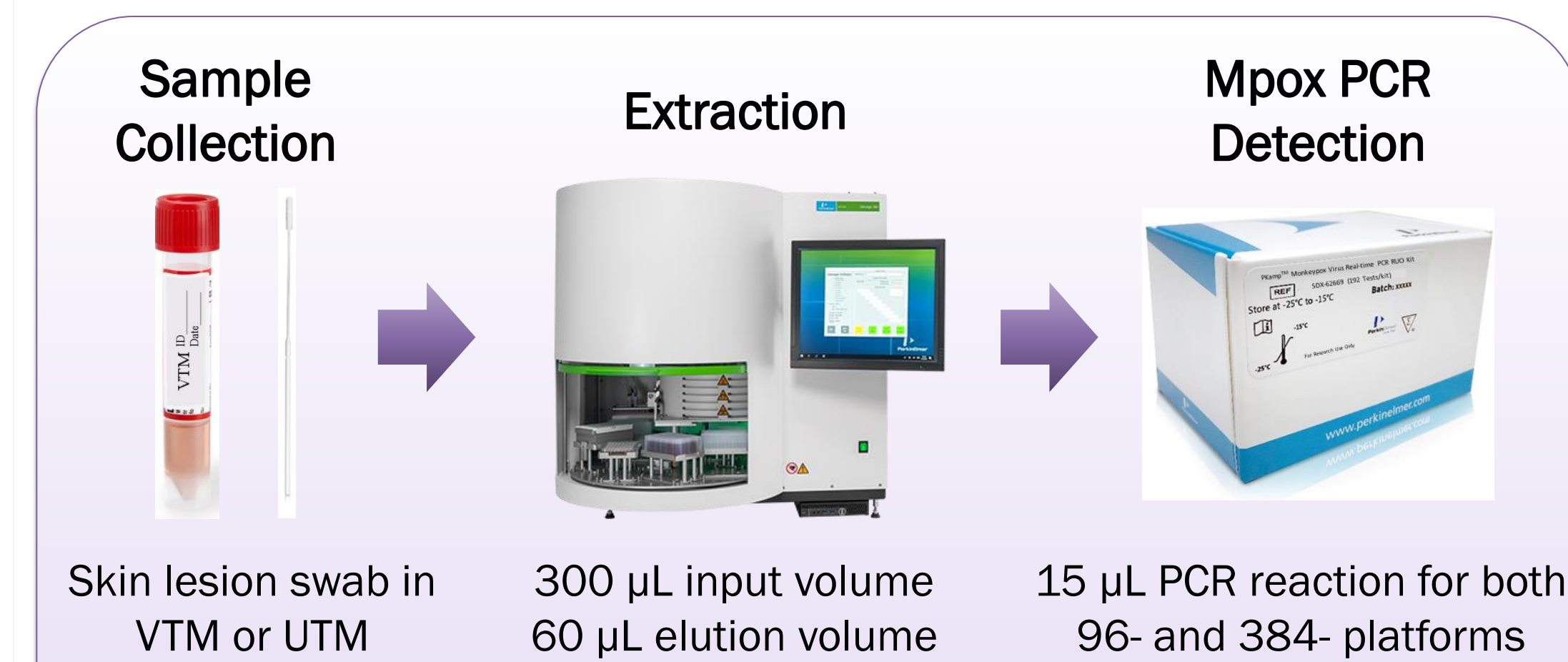
## INTRODUCTION

Mpox (formerly known as monkeypox) virus is a double stranded DNA virus that leads to rash and flu-like symptoms. It can be spread between people or between people and certain animals. Real-time PCR is a rapid method for tracking the spread of mpox which is critical for public health decision making. Here, we present a series of studies using the PerkinElmer's chemagic™ 360 system and the PKamp™ Monkeypox Virus Real-Time PCR RUO Kit, with entire workflow around 2.5 hours.

### Performed studies (selected)

- Limit of detection (LoD) confirmation
  - Inactivated mpox virus spike-in pooled mpox negative skin lesion swab VTM matrix
  - Synthetic mpox DNA spike-in simulated VTM matrix
- Matrix equivalence (VTM vs. UTM)
- Mpox inclusivity (*in silico*)
- Microbial cross-reactivity and interference
- Substance interference
- Contrived sample evaluation
- Clinical sample evaluation & comparison to the CDC reference method

## APPLICATION WORKFLOW



### RESULT INTERPRETATION

Target Channel	Mpx FAM	RNase P HEX/VIC	Result interpretation
Ct value	≤ 40	/	Mpox virus detected.
	Undet.	≤ 35	Mpox virus not detected.
	Undet.	Undet. or > 35	Invalid. Re-tested with leftover extracted DNA, or re-collection from specimen.

Undet.: undetected ; /: any Ct value

### Compatible qPCR instruments

- Applied Biosystems™ 7500 Fast systems
- Bio-Rad® CFX Touch™ systems
- EONIS™ Q systems
- QuantStudio™ systems
- qTower3/3G systems

## RESULTS

**LoD Confirmation – Pooled mpox negative skin lesion matrix with inactivated mpox virus**

All four instruments show comparable LoD at 74 cp/mL.

Instrument	Cp/mL	Mpx				RNase P			
		N (Hit) %	N (Hit)	Mean Ct	Std Dev	N (Hit) %	N (Hit)	Mean Ct	Std Dev
ABI 7500 Fast Dx	24	65%	13/20	36.95	0.83	100%	20/20	29.26	0.43
	74	100%	20/20	35.77	1.04	100%	20/20	28.86	0.49
CFX 384	24	80%	16/20	36.58	1.00	100%	20/20	25.67	0.14
	74	100%	20/20	35.44	1.02	100%	20/20	25.60	0.24
QS 5 384	24	65%	13/20	37.38	0.88	100%	20/20	27.79	0.16
	74	100%	20/20	36.28	0.79	100%	20/20	27.60	0.33
QS Dx 96	24	80%	16/20	36.76	0.86	100%	20/20	28.83	0.23
	74	100%	20/20	35.51	0.94	100%	20/20	28.56	0.22

Replicates (N) per tested condition = 20  
Inactivated virus strain: USA/MA001/2022 (ZeptoMetrix)  
Cp/mL: copies/mL  
Std Dev: standard deviation

**LoD Confirmation – Simulated VTM matrix with synthetic mpox DNA**

All four instruments show comparable LoD within 1-3 times difference.

Instrument	Cp/mL	Mpx				RNase P			
		N (Hit) %	N (Hit)	Mean Ct	Std Dev	N (Hit) %	N (Hit)	Mean Ct	Std Dev
ABI 7500 Fast Dx	74	35%	7/20	38.11	0.63	100%	20/20	30.31	1.14
	222	95%	19/20	37.21	1.12	100%	20/20	30.73	0.41
CFX 384	666	100%	20/20	35.04	0.57	100%	20/20	30.65	0.35
	222	85%	17/20	35.98	0.81	100%	20/20	29.22	0.17
QS 5 384	666	100%	20/20	34.00	0.60	100%	20/20	29.17	0.12
	222	70%	14/20	36.87	0.74	100%	20/20	30.54	0.25
QS Dx 96	666	100%	20/20	35.24	0.81	100%	20/20	30.37	0.27
	222	85%	17/20	36.16	0.73	100%	20/20	30.24	0.31
	666	100%	20/20	34.33	0.77	100%	20/20	30.15	0.24

Replicates (N) per tested condition = 20  
Synthetic mpox DNA strain: Monkeypox/PT0005/2022 (Twist Bioscience)  
Cp/mL: copies/mL  
Std Dev: standard deviation

**Matrix Equivalence – Simulated VTM vs. simulated UTM (with synthetic mpox DNA)**

VTM and UTM matrixes show equivalent sensitivity.

Instrument	Matrix	X LOD	Mpx				RNase P			
			N (Hit) %	N (Hit)	Mean Ct	Std Dev	N (Hit) %	N (Hit)	Mean Ct	Std Dev
QS 5 384	VTM	0	0%	0/10			100%	10/10	27.72	0.28
		1/3X	75%	15/20	37.21	0.77	100%	20/20	28.17	0.25
		1X	95%	19/20	35.64	0.98	100%	20/20	27.96	0.25
	UTM	0	0%	0/10			100%	10/10	27.89	0.12
		1/3X	80%	16/20	36.50	0.78	100%	20/20	28.26	0.15
		1X	95%	19/20	35.94	1.14	100%	20/20	27.74	0.23
QS Dx 96	VTM	0	0%	0/10			100%	20/20	27.77	0.18
		1/3X	80%	16/20	36.15	0.75	100%	20/20	27.42	0.24
		1X	100%	20/20	34.83	0.78	100%	20/20	28.57	0.57
	UTM	0	0%	0/10			100%	20/20	28.04	0.37
		1/3X	75%	15/20	36.05	0.6	100%	20/20	27.93	0.25
		1X	100%	20/20	35.44	0.85	100%	20/20	28.04	0.33
		3X	100%	20/20	33.22	0.51	100%	20/20	28.25	0.34

Mpx positive replicates (N) = 20; Mpx negative replicates (N) = 10  
Synthetic mpox DNA strain: Monkeypox/PT0005/2022 (Twist Bioscience)  
Std Dev: standard deviation

### Mpox Inclusivity – *in silico*

Assay inclusivity of mpox is high covering clade I and II.

Target Database	Mpox virus					
	GISAID			NCBI		
Lineage info	Clade I		Clade II		N/A	
Number of sequences	33	100%	2268	100%	1407	100%
Sequences with mutation	0	0%	1*	0.04%	1*	0.07%
Predicted loss of mpox detection	0	0%	0	0%	0	0%

Data are collected as of February 2023. Note that the GISAID sequence quality filters with "complete" is applied for clade I, and with "complete-and-high-coverage" for clade II.  
\*: One base pair (bp) mismatch on the forward primer  
N/A: not applicable

**Microbials Cross-Reactivity & Interference – Simulated VTM matrix with or without synthetic mpox DNA**

Assay cross-reactivity and interference to mpox detection are not found in the presence of individual microbial at tested concentration

#	Microorganism	Evaluated concentration	N (Hit) %			
			No Mpx		3X LoD Mpx	
			Mpx	RNase P	Mpx	RNase P
1	<i>Bacteroides fragilis</i>	10%	0%	100%	100%	100%
2	Camelpox virus	1E5 cp/mL	0%	100%	100%	100%
3	Cowpox virus	2E4 cp/mL	0%	100%	100%	100%
4	Ectromelia virus (mousepox)	1E3 pfu/mL	0%	100%	100%	100%
5	Human genomic DNA	1E6 cp/mL	0%	100%	100%	100%
6	<i>Pseudomonas aeruginosa</i>	1E6 cp/mL	0%	100%	100%	100%
7	<i>Trichophyton rubrum</i>	2E5 cfu/mL	0%	100%	100%	100%
8	Vaccinia virus (smallpox vaccine)	1E5 cp/mL	0%	100%	100%	100%

Replicates (N) per tested condition = 3  
Synthetic mpox DNA strain: Monkeypox/PT0005/2022 (Twist Bioscience)  
Cp/mL: copies/mL  
Pfu/mL or CfU/mL: plaque forming units /mL or colony forming units /mL  
QS 5 384 and QS Dx 96 were used

**Substance Interference – Simulated VTM matrix with or without synthetic mpox DNA**

Assay interference to mpox detection are not found in the presence of individual substance at tested concentration

#	Substance	Evaluated concentration	N (Hit) %			
			No Mpx		3X LoD Mpx	
			Mpx	RNase P	Mpx	RNase P
1	Abreva	7%	0%	100%	100%	100%
2	Acyclovir	7 mg/mL	0%	100%	100%	100%
3	Albumin	2.2 mg/mL	0%	100%	100%	100%
4	Benadryl cream*	0.4 mg/mL	0%	100%	100%	100%
5	Carmex*	6%	0%	100%	100%	100%
6	Casein	7 mg/mL	0%	100%	100%	100%
7	Cornstarch	2.5 mg/mL	0%	100%	100%	100%
8	Douche	7%	0%	100%	100%	100%
9	Feces	0.22%	0%	100%	100%	100%
10	Hydrocortisone cream	2%	0%	100%	100%	100%
11	KY Jelly	7%	0%	100%	100%	100%
12	Lanacane	3.5%	0%	100%	100%	100%
13	Mucin	0.06 mg/mL	0%	100%	100%	100%
14	Neosporin	0.25%	0%	100%	100%	100%
15	Seminal fluid	7%	0%	100%	100%	100%
16	Urine (Female)	10%	0%	100%	100%	100%
17	Urine (Male)	10%	0%	100%	100%	100%
18	Vagisil creme	1%	0%	100%	100%	100%
19	Whole blood in EDTA*	1%	0%	100%	100%	100%
20	Zinc Oxide ointment	7%	0%	100%	100%	100%

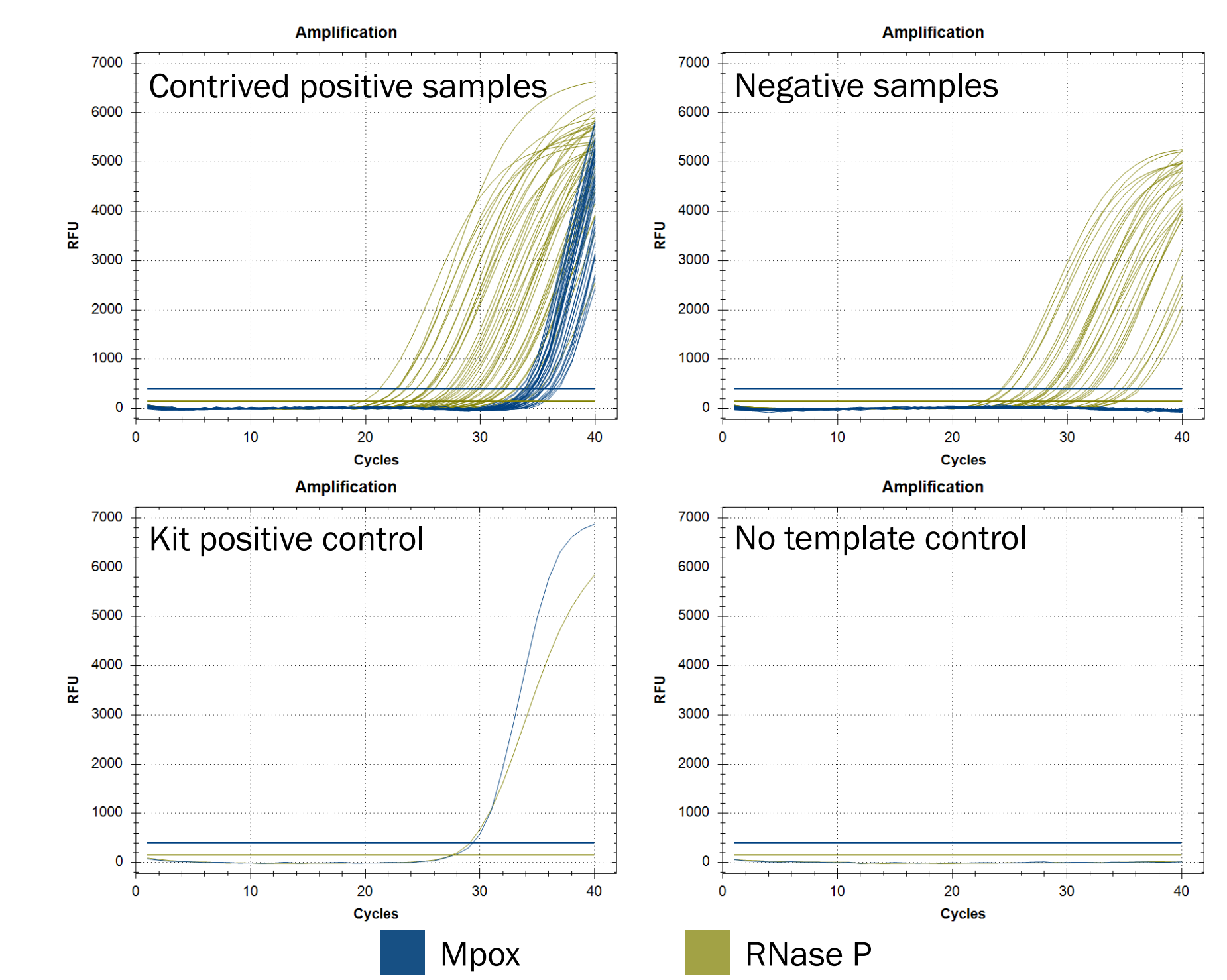
Replicates (N) per tested condition = 3  
Synthetic mpox DNA strain: Monkeypox/PT0005/2022 (Twist Bioscience)  
\*: Inhibition was observed in the presence of 0.5 mg/mL Benadryl cream, 7% Carmex, or 5% Whole blood in EDTA  
QS 5 384 and QS Dx 96 were used

**Contrived Sample Evaluation – Unique mpox negative skin lesion specimens with or without inactivated mpox virus**

Assay shows 100% agreement in contrived positive and negative samples

Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Agreement %	95% CI
						LCL, UCL
40	0	40	0	PPA	100%	91.2%, 100%
				NPA	100%	91.2%, 100%

Contrived positive samples in VTM/UTM (N) = 40 (half at 3X LoD and half at 1X LoD)  
Negative samples in VTM/UTM (N) = 40  
Inactivated virus strain: USA/MA001/2022 (ZeptoMetrix)  
CI: confidence interval  
LCL or UCL: lower control limit or upper control limit  
PPA or NPA: positive percent agreement or negative percent agreement  
CFX 96 was used



**Clinical Sample Evaluation – Assay comparison to CDC reference method**

Data show 100% agreement between the PKamp™ Monkeypox Virus Real-Time PCR RUO assay and the CDC Non-Variola Orthopoxvirus Real-Time PCR assay

Concordant Positive (N)	Discordant Negative (N)	Concordant Negative (N)	Discordant Positive (N)	Agreement Parameter	Agreement %	95% CI
						LCL, UCL
20	0	20	0	PPA	100%	83.9%, 100%
				NPA	100%	83.9%, 100%

Positive samples in VTM (N) = 20  
Negative samples in VTM (N) = 20  
ABI 7500 Fast Dx was used

## SUMMARY

- The assay reaches LoD as low as 74 cp/mL.
- The assay is compatible with VTM and UTM matrixes, and many commercial PCR instruments at equivalently sensitive level.
- The assay demonstrates 100% concordance to the CDC reference method.
- The assay uses single reaction volume (15µL) for both 96- and 384- PCR platform.
- The result is determined by Ct cutoff at end of amplification cycle.
- The entire workflow takes ~2.5 hours from sample extraction to result analysis.

## ACKNOWLEDGEMENT

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